TOXICITY OF 2,4-D AND PICLORAM TO FRESH WATER ALGAE

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Technical Paper

TOXICITY OF 2,4-D AND PICLORAM TO FRESH WATER ALGAE

TO: J. F. McLaughlin, Director
Joint Highway Research Project

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FROM: H. L. Michael, Associate Director
Joint Highway Research Project

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Attached is a short Technical Paper titled "Toxicity of 2,4-D and Picloram to Fresh Water Algae". The paper is in part from research conducted on the HPR-1 (8) research project "Research in Roadside Development", Part III Chemical Weed Control. The authors are J. H. Elder, C. A. Lembi and D. J. Morre of the Department of Botany and Plant Pathology of the School of Agriculture.

The paper is planned for presentation at the North Central Weed Control Conference and for publication in the Proceedings of that meeting to be held December 8-10, Lexington, Kentucky. The paper is presented for information and approval of such publication. It will also be forwarded to the ISHC and the FHWA for review, comment and similar approval.

Respectfully submitted,

Harold L. Michael
Associate Director

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ABSTRACT

2,4-D and picloram exhibit low toxicity to all fresh water and marine algal species thus far examined at rates approaching the maximum solubility of the herbicides in water. Use of formulated materials (especially esters) and combinations of herbicides may increase toxicity. Yet these herbicides present a low potential hazard from normal agricultural or industrial practice.

Growth regulating herbicides are used extensively for control of brush and herbaceous species in non-crop areas as well as in crop situations. In addition to the standard growth-regulating herbicides 2,4-D and 2,4,5-T, picloram has also received extensive testing for similar purposes. The mode of action of picloram is similar to that of 2,4-D (2,3) but with a greater herbicidal effectiveness due to increased mobility (3) and resistance to breakdown (5,6).

The potential hazard of Tordon herbicide to fresh water algae or phytoplankton from terrestrial runoff water or direct accidental contamination of water has received virtually no attention except from the popular press. A single report (4) describes experiments in which 1 ppm of picloram did not retard algal growth. Similarly, results with other growth regulating herbicides on growth of algae are limited.

This paper reports preliminary information on the effects of 2,4-D and picloram on growth and development of several species of freshwater and marine algae. Such information is required to evaluate the potential threat, if any, of growth regulating herbicides to our environment. Without such information, potentially useful agricultural chemicals may be lost through public or regulatory over-reaction. Alternatively, herbicides may be already entering our lakes, streams and oceans at levels dangerous to their productivity.

MATERIALS AND METHODS

Picloram (4-amino-3,5,6-trichloropicolinic acid, 99% analytical grade), technical picloram (4-amino-3,5,6-trichloropicolinic acid, 91%

technical grade) and 2,4-D (2,4-dichlorophenoxyacetic acid, 99%; biological grade) were added directly to the growth media. Fresh water algae were grown on at least one optimal medium as well as on at least one minimal medium (soil water, (7) pond water or both) under fluorescent lights (150 ft.-c) on a 16 hr photoperiod with gentle shaking. Marine organisms were cultured in an equal mixture of filtered sea water and soil water.

Growth was determined from changes in optical density at 660mμ or from cell counts. Cells were examined with the light microscope for morphological changes and if morphological changes were detected with the electron microscope.

RESULTS AND DISCUSSION

The solubility of 2,4-D acid in water is approximately 2.5×10^{-3}M, that of picloram, 1.8×10^{-3}M. Therefore, from a practical standpoint, the highest concentration tested was 10^{-3}M (220 ppm 2,4-D and 240 ppm picloram). At this concentration, we could observe no effect on most of the organisms with 2,4-D (Table 1) and none of the organisms with picloram (Table 2). In single experiments with Chlorella and Pediastrum, inhibition of growth was noted at 10^{-3}M 2,4-D but in these same experiments, growth was normal at 10^{-4}M (22 ppm). Many of the organisms were cultured for periods of several weeks in the presence of the herbicides with no harmful effects and 1 week was chosen as the minimum test period.

With technical picloram, non-motile species were generally unaffected

at the highest concentrations tested but motile species were found to lose motility at 10^{-3}M and 5 x 10^{-4}M but not at 10^{-4}M (24ppm). These results are summarized in Table 2 and data for Euglena gracilis is given in Table 3. The toxic principle is an impurity in the technical picloram tentatively identified from samples provided by the Dow Chemical Company as 2-(3,4,5,6-tetrachloro-2-pyridyl) guanidine. It should be emphasized that not all organisms were affected by technical picloram even at high concentrations. One organism, Pediastrum, grew quite well on technical Tordon. Particularly in the declining phase of the culture, more colonies were found in the presence of picloram than in its absence or in the presence of 2,4-D (Table 4).

The results show that the potential hazard of 2,4-D or picloram to both fresh water and marine algae from terrestrial runoff water (concentrations of 0.1 ppm or less) or direct or accidental contamination (3 lb/A applied directly to 6" of water gives a concentration of about 2 ppm) is nil. Further studies will be necessary to determine what effects these herbicides might have on subtle ecological shifts as well as to assess the effect of these herbicides in combination with other water pollutants. It is quite apparent from preliminary studies with formulated materials and the data of Butler (1), that certain 2,4-D derivatives (particularly esters) may be substantially more toxic than the parent acid. In this regard, it will be necessary to examine a number of 2,4-D derivatives in different formulations to seek those which will result in minimum damage to algae, fish and other aquatic organisms since the parent herbicide is nontoxic. There is no evidence for biological magnification of either 2,4-D or picloram in algae (4).

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Table 1. Response of algae to 2,4-D (< 99% acid)

Organisms not killed by 1 week exposure at 10^{-3}M (220 ppm)			
Hydrodictyon	Volvox	Oscillatoria	Coccolithus
Zygnema	Pandorina	Microcoleus	Pleurochrysis
Spirogyra	Euglena	Chroococcus	
Pithophora		Nostoc[a]	
	Navicula[a]	Cylindospermum[a]	
		Merismopedia[a]	

Organisms not killed by 1 week exposure at 10^{-4}M (22 ppm)
Chlorella
Pediastrum

[a] Single determination

Table 2. Response of algae to picloram (99% acid)

Organisms not killed by 1 week exposure at 10^{-3}M (240 ppm)				
Hydrodictyon	Chlorella	Volvox[a]	Oscillatoria[c]	Coccolithus[a]
Zygnema	Pediastrum	Pandorina[a]	Lyngbya	Pleurochrysis[b]
Spirogyra		Euglena[a]	Microcoleus	
Pithophora	Characiopsis		Chroococcus[c]	

[a]Loss of motility followed by death with Technical (91% acid) picloram.

[b]Effect of technical (91% acid) picloram most pronounced on motile zoospores.

[c]Loss of pigmentation with technical (91% acid) picloram and loss of movement of Oscillatoria with technical (91% acid) picloram.

Table 3. Effect of herbicides on Euglena gracilis, Z strain growing in soil water after 3 days

Herbicide	Conc.	O.D. 660 mμ	% Motile Cells
None	-	0.26	100
2,4-D Acid (< 99%)	10^{-3}M	0.27	100
Analytical Picloram (99% Acid)	10^{-3}M	0.26	100
Technical Picloram (91% Acid)	10^{-3}M	0.23	5
	5×10^{-4}M	0.23	10
	10^{-4}M	0.26	100

Table 4. Effects of herbicides on viability of Pediastrum in pond water

Herbicide	Conc.	Cells/ml ± Standard Deviation After 1 Doubling	Cells/ml ± Standard Deviation 2 Weeks Later
None	-	114± 14	26 ± 8
2,4-D Acid (>99%)	10^{-4}M	104± 10	26 ± 10
Technical Picloram (91% Acid)	10^{-4}M	112± 16	60 ± 10